Blood Dyscrasia in Calves Induced by S-(Dichlorovinyl)-L-Cysteine

By M. O. Schultze, P. Klubes, V. Perman, N. S. Mizuno, F. W. Bates and J. H. Sautter

In their search for the toxic factor in trichloroethylene-extracted soybean oil meal which can induce fatal aplastic anemia in calves, cattle, and horses, McKinney et al. synthesized S-(dichlorovinyl)-L-cysteine (DCVC). They reported that the oral administration of this compound to calves produced leukopenia, thrombocytopenia, a relative lymphocytosis and a hemorrhagic syndrome, conditions which are also observed when sufficient amounts of trichloroethylene-extracted soybean oil meal are fed to calves. Although the toxic factor in this meal, which elicits this syndrome, has not been isolated and characterized, its chemical properties, insofar as they have been ascertained, are consistent with the view that it may be produced through the interaction of trichloroethylene with cysteine residues of proteins of the soybean.

In conjunction with our other studies on bovine aplastic anemia, we have synthesized DCVC and confirmed its high toxicity for calves.

Experimental

Synthesis of DCVC.—L-cystine (0.075 M), [α]25D = -212° (1 per cent in N HCl), recrystallized from dilute ethanol at pH 4.4, was dissolved in liquid ammonia (300 ml.) and cooled in a bath of trichloroethylene-solid-carbon-dioxide. The cystine was reduced by the addition, in small increments, of sodium (0.30 M), with stirring. The solution of disodium cystinate was added, in small increments, to a well stirred solution of freshly distilled (B.P. 87.2° C.) trichloroethylene (0.15 M), dissolved in liquid ammonia (400 ml.) and cooled in a trichloroethylene-solid-carbon-dioxide bath. The cooling bath was then removed, and the ammonia evaporated spontaneously overnight.

After evacuation at the waterpump for 1 hour, the solid residue was dissolved in distilled water (750 ml.), filtered through a thin layer of charcoal and adjusted to pH 5.0 with acetic acid. After standing overnight at 4° C., the white precipitate was filtered off, washed with small portions of ice-cold water and dried in vacuo over sulfuric acid. This crude product was recrystallized from a hot aqueous solution by addition of 95 per cent ethanol. The yield of this white product, after drying in vacuo over sulfuric acid was, on the average 43 per cent, calculated from the cystine used. M. P., corrected, 160-161° C., with...
decomposition. Found: C, 27.9; H, 3.27; N, 6.52; S, 14.9; Cl, 32.7 per cent; calculated for \( \text{C}_2\text{H}_7\text{O}_{0.5} \text{N}_5.5 \text{Cl}_9 \): C, 27.29; H, 3.27; N, 6.48; S, 14.84; Cl, 32.82 per cent.

Animal Experiments.—Female Holstein calves weighing about 45 Kg. were purchased in the open market. They were managed as previously described and conditioned for a pretreatment period of about two weeks. They received a ration of whole cow’s milk and alfalfa hay and had access to a mineralized salt block. Before and during the trial, blood was withdrawn from a jugular vein at frequent intervals, daily when the dosage of DCVC was 2.2 mg. or more per Kg. Standard methods were used for determination of hematocrit, hemoglobin, total and differential white cell counts and thrombocyte counts.

DCVC was dissolved with slight warming in 0.9 per cent sterile sodium chloride solution (4 to 5 mg. per ml.) and injected into a jugular vein daily for 10 consecutive days or, in some instances, for only one day; with daily dosage of 2.2 mg. or greater per Kg. of body weight, the compound was injected in three equal portions at 4 hour intervals.

Six control animals received daily intravenous injections of 0.9 per cent sodium chloride, corresponding in volume to the injected DCVC solutions.

### RESULTS AND DISCUSSION

The results summarized in table 1 demonstrate that as little as 0.33 mg. of DCVC per day per Kg. of body weight injected daily for 10 days can be fatal to young calves. As in the case of trichloroethylene-extracted soybean oil meal, continuous administration of DCVC is not necessary, either to produce the blood dyscrasia or to cause death.

**Blood.**—Figures 1 and 2 illustrate the changes in the blood picture induced by amounts of DCVC ranging from 0.44 to 2.2 mg. per Kg. body weight. In all cases, thrombocyte counts remained in the normal range for about 10 to 14 days. Thereafter, they decreased rapidly until counts of as little as 10,000 per cu. mm. of blood were observed.

With the lower dosages (fig. 1), the leukocyte count remained essentially constant until about the 11th day when it decreased rapidly. This decrease was initially due mainly to a rapid disappearance of the polymorphonuclear neutrophils.

### Table 1.—Toxicity of S-(dichlorocinyl)-L-cysteine for Calves

<table>
<thead>
<tr>
<th>Number of calves</th>
<th>Dosage Mg./Kg./day</th>
<th>Number of days injected</th>
<th>Deaths on day</th>
<th>Number of survivors</th>
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<tr>
<td>2</td>
<td>6.7</td>
<td>1</td>
<td>1st,5th</td>
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</tr>
<tr>
<td>1</td>
<td>5.0</td>
<td>1</td>
<td>6th</td>
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</tr>
<tr>
<td>4</td>
<td>4.4</td>
<td>1</td>
<td>6th,7th,9th,20th</td>
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</tr>
<tr>
<td>1</td>
<td>4.1</td>
<td>1</td>
<td>20th</td>
<td>none</td>
</tr>
<tr>
<td>3*</td>
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<td>4,8,10</td>
<td>4th,8th,10th</td>
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<tr>
<td>2*</td>
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<td>5,10</td>
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</tr>
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<td>10</td>
<td>16th,18th</td>
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<td>2</td>
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<td>10</td>
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</tr>
<tr>
<td>3</td>
<td>0.33</td>
<td>10</td>
<td>none</td>
<td>3</td>
</tr>
<tr>
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<td>0.22</td>
<td>10</td>
<td>none</td>
<td>3</td>
</tr>
<tr>
<td>6†</td>
<td>0</td>
<td>10</td>
<td>none</td>
<td>6</td>
</tr>
</tbody>
</table>

*Injected daily until death.
†Living on 96th day; slaughtered.
‡Control animals injected for 10 days with 0.9% NaCl.
§Since submission of this manuscript four calves have been injected intravenously with 3.3 mg. DCVC per Kg. body weight given on one day. They died 23 to 27 days later.
leukocytes followed later by a decrease in the lymphocyte count. During the last four days, the lymphocytes accounted for all of the white cells. In the terminal stages of the intoxication there was a marked decrease of the hemoglobin, mainly due to internal hemorrhage.

When the dosage of DCVC was 2.2 mg. per day per Kg. of body weight the decreases of the total white count, the polymorphonuclear leukocytes and the lymphocytes preceded the decrease in thrombocytes. The number of lymphocytes remained relatively constant until about the sixth day. However, after two injections, there was a temporary three- to fourfold increase of the polymorphonuclear leukocytes. A similar, but even more pronounced
Fig. 2.—Hematologic changes induced by intravenous injections of 2.2 mg. DCVC per Kg. body weight per day for 10 days. Mean values from two calves.

Granulocytosis has also been observed in the three calves which were injected with 3.3 or 4.4 mg. of DCVC per Kg. body weight per day (fig. 3). This temporary granulocytosis, occurring shortly after treatment with an agent which ultimately leads to complete inhibition of hematopoiesis, simulates the effects of X-rays in other species or of gamma rays in calves.

Calves had a poor tolerance for DCVC when it was injected intravenously in amounts of about 4 mg. or more per Kg. of body weight, as shown in table 1. Two calves were injected with 6.7 mg. per Kg. body weight in four divided doses during a period of 8 hours; one of these animals was dead 24 hours after the first injection; the other developed complete anuria and died five days later. One calf, similarly treated with 5.0 mg. and three others with
44 or 33 mg DCVC/day/kg. LV.

Leukocytes

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C

0

F-

a)

C-

a)

C

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S

S

I 41...

....&..

0....,

Lymphocytes

-1 0

8 10

DICHLOROVINYL-L-CYSTEINE IN CALVES

Fig. 3.—Hematologic changes induced by intravenous injections of 3.3 or 4.4 mg. DCVC per Kg. body weight per day for 10 days. Mean values from two calves, one on each dosage. The time course of the changes was essentially the same in both calves.

4.4 mg. DCVC for only one day, died in 5 to 9 days, all showing signs of severe kidney damage at necropsy. However two calves injected with 4.4 and 4.1 mg. DCVC respectively on one day survived for twenty days. Both developed fatal aplastic anemia as shown in figure 4. In these animals, the leukopenia developed less rapidly than when a similar amount of DCVC was administered daily. The data in figure 4 show clearly, however, that hematopoietic activity of the bone marrow can be completely blocked by administration of DCVC during a few hours.

Figure 5 illustrates the hematologic changes (mean values) in two calves which were injected intravenously for 10 days with 0.22 mg. DCVC per day per Kg. body weight. Like the calves treated with larger amounts of the toxic compound, they developed severe thrombocytopenia, leukopenia and
Fig. 4.—Mean hematologic changes induced by intravenous injection into two calves of 4.4 or 4.1 mg. DCVC per Kg. body weight on day 0. Both animals died on day 20.

granulocytopenia. These animals survived, however, and made a gradual recovery, the thrombocyte counts reaching about 500,000/cu.mm. and the leukocyte counts about 9,000/cu.mm. by the 90th day. Another calf treated with 0.22 mg. of DCVC per Kg. per day for 10 days developed a similar blood dyscrasia at the same time, but her recovery was more rapid. Of three calves treated for 10 days with 0.33 mg. DCVC per day per Kg. body weight, two died on the 23rd and 34th day after developing hematologic changes similar in sequence and severity to the cases illustrated in figure 1; another survived for 90 days following a course of blood changes essentially as shown in figure 5.

The high susceptibility of the calf to DCVC is in marked contrast to the
relative resistance of the hematopoietic and other tissues of the rat to this compound.17

Bone marrow.—Judging from the quantitative changes in the circulating blood cells after administration of DCVC, we expected an early depletion of their precursors in the bone marrow. This was confirmed by examination of biopsy specimens. The rate and extent of this depletion is related to the amounts of DCVC administered.

Figures 6 A–C illustrate the appearance of stained smears of bone marrow removed from the same calf by sternal biopsy before, 15 days and 21 days after the first intravenous injection of 0.66 mg. DCVC per day per Kg. In
contrast to the control specimen, only a few immature forms of the erythroid, myeloid and lymphoid series of cells were still present on the 15th day, and these disappeared almost completely by the 21st day. When the dosage of DCVC was increased to 4.4 mg per day per Kg., severe hypoplasia of the bone marrow was apparent on the 6th and 8th days after the first injections, in those animals which survived for these periods (fig. 7 A–C). Smears prepared at intervals from sternal bone marrow of control calves injected with 0.9 per cent sodium chloride revealed no decrease of the high density of immature precursors of blood cells in various stages of development. The blood dyscrasia induced by DCVC is therefore the result of an inhibition of the hematopoietic activity of the bone marrow which appears
to be irreversible when the daily dosage is about 0.4 mg. per Kg. (see table 1).

Hemorrhagic syndrome.—All calves that became moribund after treatment with 0.33 to 2.2 mg. DCVC per day per Kg. experienced a severe hemorrhagic syndrome. The lesions ranged in size from petechial and ecchymotic foci to massive areas of involvement. The latter were found particularly in the lungs, the thoracic pleura and the mucosal and serosal surfaces of the intestine. In contrast, no hemorrhagic lesions were found in the calves which were treated with 3.3 or 4.4 mg. DCVC per day per Kg. and became moribund.
in about 10 days or less (table 1). However, the calves which lived for 20 days after treatment with about 4 mg. of DCVC on one day only developed the typical hemorrhagic syndrome of aplastic anemia.

A detailed account of the gross and microscopic pathologic findings on the calves will be published elsewhere.

CONCLUSIONS

1. S-(dichlorovinyl)-L-cysteine was synthesized and its high toxicity for calves confirmed.

2. Intravenous injection of 0.22 mg. of this compound per day per Kg. body weight for 10 days was sufficient to induce severe hypoplasia of the bone marrow, and a blood dyscrasia which reached its greatest severity between the 25th and 30th day after the start of treatment, followed by gradual recovery. The injection of 0.33 to 2.2 mg. per day per Kg. of body weight for 10 days produced thrombocytopenia, leukopenia, lymphopenia and a fatal hemorrhagic syndrome.

3. Intravenous injection of about 4 mg. S-(dichlorovinyl)-L-cysteine per Kg. body weight on one day may result in death of calves from causes other than hematopoietic failure. However, if the animals survive the acute toxic effects, this amount is sufficient to block hematopoietic activity and to cause death from aplastic anemia about three weeks later.

SUMMARIO in INTERLINGUA

1. S-(dichlorovinyl)-L-cysteina esseva synthetisate. Su alte toxicitate pro vitallos esseva confirmate.

2. Injectiones intravenose de 0,22 mg del composito per die per kg de peso corporee durante 10 dies sufficéva pro inducer grados sever de hypoplasia del medulla ossea e un dyscrasia del sanguine que attingeva su maximo de severitate inter le vinti-quinete e le trentesime die post le initiation del tractamento. Isto esseva sequite per un restablimento gradual. Injectiones intravenose de inter 0,33 e 2,2 mg del composito per die per kg de peso corporee durante 10 dies induceva thrombocytopenia, leucopenia, lymphopenia, e un mortal syndrome hemorrhagic.

3. Le injection intravenose de circa 4 mg de S-(dichlorovinyl)-L-cysteina per kg de peso corporee in un sol die pote resultar in le morte de vitellos ab causas altere que insufficientia hematopoietic. Tamen, si le animales supervive al acute effectos toxic del composito, le quantitate mentionate suficé pro blocar le activitate hematopoietic e pro causar le morte ab anemia aplastic circa tres septimanas plus tarde.

REFERENCES


9. ——.: Personal communication.


17. Unpublished observations by the authors.
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